PROTOCOL

Standardised treatment and monitoring protocol for adult and paediatric patients receiving bacteriophage therapy

Short title: Standardised Treatment and Monitoring of Phage therapy (STAMP)

Version: 1.1

Date: 15/07/2022

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

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ADMINISTRATIVE INFORMATION

1. TITLE

Standardised treatment and monitoring protocol for adult and paediatric patients receiving bacteriophage therapy

Short title: Standardised Treatment and Monitoring of Phage therapy (STAMP)

2. TRIAL REGISTRATION

2A REGISTRY

anzctr.org:

2B DATA SET

Data Category	**Information**
Primary registry and study identifying number	anzctr.org ACTRN12621001526864
Date of registration in primary registry	10 November 2021
Secondary identifying numbers	WHO UTN: U1111-1269-6000
Source(s) of monetary or material support	Medical Research Futures Fund (Australia) Frontiers Stage 1 (RFRHPI000017; Iredell) National Health and Medical Research Council (Australia) Investigator Grant (APP1197534; Iredell)
Primary Sponsor	Western Sydney Local Health District
Contact for public queries	Ameneh Khatami (ameneh.khatami@health.nsw.gov.au)
Contact for scientific queries	Ameneh Khatami (ameneh.khatami@health.nsw.gov.au)

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Data Category	**Information**
Public title	Standardised Treatment and Monitoring of Phage therapy (STAMP)
Scientific title	Standardised treatment and monitoring protocol for adult and paediatric patients receiving bacteriophage therapy
Countries of recruitment	Australia
Health condition(s) or problem(s) studied	Any infectious syndrome requiring bacteriophage therapy
Intervention(s)	A standardised treatment and monitoring protocol for bacteriophage therapy
Key inclusion and exclusion criteria	**Inclusion criteria (all must be present):** 1) Patient with confirmed bacterial infection, for whom at least **two** appropriately qualified clinical specialists, including at least one specialist in infection management and one with experience of phage therapy, have agreed that available anti-infective therapy and source control has been optimised and that adjunctive phage therapy should be offered. 2) A suitable phage(s) product is available that complies with all relevant regulatory requirements for therapeutic administration, including at least Therapeutic Goods Administration (TGA) Special Access Scheme (SAS) notification **Exclusion criteria:** 1) Participant or person responsible has not provided informed consent 2) Participant unable or unlikely to adhere to schedule of monitoring and follow-up
Study type	Open-label, single arm trial of a standardised treatment and monitoring protocol
Date of first enrolment	April 2022
Target sample size	50-100 participants over 5 years

Data Category	**Information**
Primary outcome	Safety and tolerability of phage therapy (defined as absence serious adverse events attributable to phage therapy) assessed 15 days after completion of therapy
Key secondary outcomes	1) Long-term safety and tolerability of phage therapy (defined as absence of serious adverse events, or any adverse events attributable to phage therapy) assessed 6 months after completion of phage therapy. 2) Feasibility of a standardised protocol as assessed by the proportion of participants with >80% of Minimum Data Requirements available for analysis. **Exploratory outcomes (assessed across the total cohort and subgroups of participants):** 1) Clinical response to phage therapy assessed 15 days after completion of therapy 2) Patient satisfaction of outcome assessed at multiple time-points during and after completion of therapy 3) Microbiological clearance of target pathogen(s), as defined by bacterial cultures of relevant sites a. Proportion with documented clearance and time to clearance b. Emergence of anti-phage resistance on treatment 4) Laboratory markers of phage and host responses, including: a. Phage pharmacokinetics b. Antibody development c. Host immune responses 5) Changes in microbiome assessed by metagenomics during and after completion of therapy

3. PROTOCOL VERSION

Issue Date:	**Version 1, 20 December 2021**
Protocol amendment number:	**N/A**
Author(s):	**Ameneh Khatami** **Jonathan Iredell** **Steven Tong** **Morgyn Warner** **Anton Peleg** **Thomas Snelling** **Julia Warning (NSW Ministry of Health)** **Elizabeth Barnes (independent statistician)** **Phage Australia Clinical Stream Committee members**

Revision Chronology:

Khatami A, *et al. BMJ Open* 2022; 12:e065401. doi: 10.1136/bmjopen-2022-065401

Date of change	Summary of changes
Version 1.1 29 May 2022	Section 2B (Registry Data Set): first participant enrolled April 2022, not Jan 2022. Date of registration and registry identifier added. Section 3: Version 1 issue date 20/12/21, not 30/09/21; and addition of Dr Julia Warning to the author list (previously missed in error) Section 5D: Clarification that additional recruitment sites and laboratories may be added during the trial, with new PIs added to the Investigator Group Section 11.4: Administered doses corrected from AT LEAST 10^9 PFU/mL (IV) or 10^{10}PFU/mL non-IV, to APPROXIMATELY 10^9 PFU/mL (IV) or 10^{10}PFU/mL non-IV Section 12 (microbiological endpoints): clarification that "Time to clearance" will be measured for each site of infection. Figure 1: Clarification in footnotes that patients receiving longer courses of phage therapy will have an additional quality-of-life survey at 12 months after completion of therapy, as outlined elsewhere in the protocol Section 26: Clarification that repeated courses of treatment in the trial will only be described but not included in the primary analyses (as outlined elsewhere in the protocol). Throughout: All referenced to 2 weeks changed to either 14 days (treatment) or 15 days (follow-up) as appropriate, and one month replaced with 29 days to remove ambiguity about durations, in line with information outlined in table 2/3 and figure 1.

4. FUNDING

Initial set-up costs for the trial, including database design and development and project management, are funded by a Medical Research Futures Fund (MRFF) Frontiers Stage 1 grant (RFRHPI000017; PHAGE AUSTRALIA: Integrating Australian Phage Biobanking and Therapeutic Networks and Delivering Solutions for Antimicrobial Resistance, CIA Iredell).

Local phage product development including Good Manufacturing Practice (GMP)-grade therapeutic products, and development of companion diagnostics are supported by the MRFF Frontiers Stage 1 grant, and a National Health and Medical Research Council Investigator Grant (GNT1197534: Positive Solutions for Critical Infection, CIA Iredell).

Phage and companion diagnostics support may come from research and professional partners, provided they are fit for purpose and satisfy all national, state and local institutional requirements as determined by authorities responsible for clinical and research governance and for regulating the provision and quality control of diagnostic and therapeutic goods.

Patients included in this trial may receive therapeutic phage products sourced from industry partners/commercial suppliers provided on "compassionate grounds".

All investigators provide their time and expertise as in-kind contributions without payment. Local Health Districts (LHDs) and Health Networks cover the costs of inpatient and outpatient care of patients according to usual standards.

5. Roles and Responsibilities

5A Contributorship

AK and JI conceived the study. AK, JI, ST and MW initiated the study design and protocol development. JI is a grant holder. TS and EZ provided statistical expertise. All authors contributed to refinement of the study protocol.

5B Sponsor Contact Information

Study Sponsor	Western Sydney Local Health District
Contact name	Sharon Lee
Address	WSLHD Research and Education Network, Clinical Trials Support Unit, Westmead Hospital, Hawkesbury Road, Westmead NSW 2145
Telephone	02 8890 9942,
Email	WSLHD-ClinicalTrialsSupportUnit@health.nsw.gov.au

5C Sponsor and Funder

The study Sponsor delegates responsibility for study design; collection, management, analysis, and interpretation of data; writing of the report and the decision to submit the report for publication to the CPI and the Trial Steering Committee (TSC). Any funding body involved in the trial will not have any role in these responsibilities.

5D Committees

1. Investigator Group
    a. Co-ordinating Principal Investigator: Jonathan Iredell
    b. Principal Investigators (PIs) for clinical recruitment sites
        i. Westmead Hospital: Jonathan Iredell
        ii. Sydney Children's Hospitals Network: Ameneh Khatami
        iii. The Alfred Hospital: Anton Peleg
        iv. Royal Melbourne Hospital: Steven Tong
        v. Central Adelaide Local Health Network: Morgyn Warner
        vi. Royal Brisbane and Women's Hospital: David Paterson
        vii. Queensland Children's Hospital: Adam Irwin

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viii. Fiona Stanley Hospital, Murdoch: Christopher Heath
ix. Perth Children's Hospital: Stephen Stick
x. Additional sites may be added during the course of the trial with new PIs added to the Investigator Group

c. Principal Investigators (PIs) for research institutes and laboratories where participant samples may be processed
i. Westmead Institute for Medical Research: Jonathan Iredell
ii. Monash Centre to Impact Antimicrobial Resistance: Anton Peleg
iii. Telethon Kid's Institute: Stephen Stick
iv. The Doherty Institute: Deborah Williamson
v. Additional sites may be added during the course of the trial with new PIs added to the Investigator Group

2. The TSC will be the Clinical Stream Steering Committee of Phage Australia, a multidisciplinary group that, collectively, have experience/expertise in the management of patients with condition(s) relevant to the study, pathways for use of phage therapy, anticipated adverse events, and in the conduct and monitoring of randomised clinical trials. Co-conveners are Steven Tong (Victoria), Ameneh Khatami (New South Wales [NSW], paediatrics), Morgyn Warner (South Australia). The committee also includes members with statistical expertise and stakeholder representatives including consumer representatives and NSW Ministry of Health representatives. Details of membership and specified roles and responsibilities are outlined in the Terms of Reference for this Committee.

3. A Data Safety Monitoring Board will include a chair with management experience in clinical trials and 5 additional members, of whom at least 1 will be paediatrician, at least 1 will be an adult physician and at least 1 will have established clinical expertise in the delivery of phage therapy. Additional details of membership and specified roles and responsibilities are outlined in the Terms of Reference for the DSMB. The DSMB will have access to all safety data available at the time of each meeting. They will be asked to review the data and make a recommendation to the CPI and site PIs within 4 weeks of the meeting. Review of the safety data may result in a recommendation for:
   - Cessation of the trial for all participants or for a subgroup (e.g. only paediatric participants, participants treated for a specific indication, or participants from a given site)
   - Interim suspension while reviewing the data in more detail
   - Modification of the trial
   - Continuation of the trial

INTRODUCTION

6. BACKGROUND AND RATIONALE

Antibiotics are now failing due to growing antimicrobial resistance (AMR) which at least doubles mortality and health care costs of infection. Without novel solutions, AMR infections are predicted to be the leading cause of death by 2050, cost US$30 billion annually in lost productivity of the global

economy, and threaten many advances in medicine(*1*). A key unmet need is for new strategies to manage difficult-to-treat infections, including chronic infections in cystic fibrosis (CF), other infections that respond poorly to antibiotics (e.g. prosthetic device infections) and highly drug resistant infections.

Bacteriophages (phages) are viruses that naturally infect and kill bacteria. Phages prey equally well on antibiotic-sensitive and -resistant bacteria and offer a novel (non-antibiotic) approach to treat infections. Unlike antibiotics that kill both disease-causing and beneficial bacteria leaving the patient vulnerable to other pathogens(*2*), the highly selective bacterial killing by phages reduces their impact on our healthy microbiome(*3*), as well as the risk of further increasing drug resistance. Phages do not attack human cells and phage therapy has a proven safety record, offering hope for people with highly drug resistant infections(*4-10*). Other critical advantages over antibiotics include the ability of phages to kill bacteria in biofilms (bacteria that hide away in a layer of slime, often on artificial surfaces) when antibiotics typically cannot(*11*), since phages are unaffected by the mechanisms that shield bacteria in biofilms from antibiotics(*12*). Biofilms are present in a wide variety of human infections and are usual in CF and on infected prosthetic devices and artificial heart valves(*13*).

Phages can be used in combination with antibiotics or substitute for them when antibiotics fail and can even be used to restore the potency of failing antibiotics(*14-16*). **Currently, phage therapy is not in wide use because there is no experience outside of a few centres in the world; standardised therapeutic protocols are lacking; there is a paucity of rigorous clinical trial data assessing efficacy and phages are generally not readily available in sufficiently purified form for use.** We and others(*5*) have built on a century of international experience to prove the value of phage therapy after antibiotic failure in severe sepsis(*6, 8*), bladder(*9*) and bone infection(*10*), and in CF [manuscript in preparation]. Our group were the first in the world to use intravenous (IV) phages in a systematic trial in severe infection in adults and thus gained new insights into dosing, disposition in the body (kinetics of distribution and clearance) and effects on the immune system(*8*). In parallel, we have established a program for compassionate access to phage therapy for children at Sydney Children's Hospitals Network (SCHN) and this protocol represents a national expansion of this programme for adults and children, providing a standardised treatment and monitoring protocol, and linked to a national database to facilitate standardised data collection. This will allow rapid and efficient data generation without wastage of a currently "scarce" resource, and will strengthen the evidence base, including for dosing and monitoring, required to establish phage therapy in the national formulary.

A standardised treatment and monitoring protocol for Australian adult and paediatric patients receiving bacteriophage therapy is proposed, with no comparator arm. Patients included are those who have exhausted other therapeutic options for control of their infection and are being treated with phage products according to the special access scheme (SAS) as determined by the Australian Therapeutic Goods Administration (TGA) – so-called "compassionate access". Patients who are assessed to be suitable for phage therapy will receive this as outlined in this protocol, as an adjunct to routine clinical care. **The purpose of the study is to standardise therapeutic management and data collection, including safety and tolerability, in this setting and to assess the feasibility of such a standardised protocol.**

7. STUDY OBJECTIVES

a. **Primary Objective**: To determine the short-term safety and tolerability of phage therapy in adults and children with bacterial infections

b. **Secondary Objectives**:

   i. To determine the long-term safety and tolerability of phage therapy in adults and children with bacterial infections

   ii. To assess the feasibility of a standardised protocol used for the administration and monitoring of phage therapy in adults and children

c. **Exploratory Objectives:**

   i. To describe proportions of patients with good clinical response to phage therapy, assessed 15 days after completion of therapy, across the cohort of participants and according to clinical indication

   ii. To describe patient-reported quality-of-life indicators during and after phage therapy across the cohort of participants and according to clinical indication using a validated Patient Reported Outcome Measures (PROMs) tool (EQ-5D-5L/EQ-5D-Y)

   iii. To describe proportions of patients with microbiological clearance, and time to clearance, across the cohort of participants and according to clinical indication

   iv. To describe the rate of phage resistance emergence while on therapy

   v. To explore pharmacokinetics (distribution and clearance kinetics) of phages administered via different routes and according to different dosing schedules

   vi. To explore innate and adaptive immune responses to phage therapy (include age-related differences) administered via different routes and for different clinical indications

   vii. To explore optimal dosing schedules and durations for phage products used according to different routes of administration (IV, oral, nebulised/aerosolised, topical) for different clinical indications

   viii. To explore key biomarkers that can be used as diagnostic surrogates of clinical efficacy

   ix. To explore significant differences between different phage products used for the same clinical indication with respect to phage pharmacokinetics, innate and adaptive immune responses, safety and tolerability and clinical efficacy

   x. To explore microbiome changes as assessed by non-human metagenomics of available clinical samples during and after completion of phage therapy.

8. STUDY DESIGN

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Open-label, single-arm trial investigating a standardised treatment and monitoring protocol for phage therapy, with multiple subgroups stratified according to clinical indication and route of administration of phages (see section 11A – Interventions).

METHODS: PARTICIPANTS, INTERVENTIONS, OUTCOMES

9. STUDY SETTING

Participants can be recruited from any hospital (public or private) in Australia, where ethics and site-specific approval has been gained. Participants can be treated as inpatients or outpatients.

9.1 Study sites

Study sites will be selected based on:

1) Access to appropriate facilities and staff to undertake the full treatment and monitoring protocol according to all regulatory approvals (e.g. physical containment [PC] level 2 facilities for use of a GMO phage, if applicable)
2) An identified site PI with experience in phage therapy or infection management.
3) A site feasibility survey is completed and approved by the Trial Steering Committee

10. ELIGIBILITY CRITERIA

10.1 **Inclusion Criteria for Participants** (all must be present):

1. Patient with confirmed bacterial infection, for whom at least **two** appropriately qualified clinical specialists, including at least one specialist in infection management and one with experience of phage therapy, have agreed that available anti-infective therapy and source control has been optimised and that adjunctive phage therapy should be offered (see section 11.2 below). The assessment of the need for/suitability for phage therapy will be based on clinical criteria and will not constitute a research activity.
2. Suitable phage(s) product (with lytic activity against the target pathogen/s) is available that complies with all relevant regulatory requirements for therapeutic administration. These requirements may include:
   i. Local hospital/network drug committee and/or Executive board approval (or equivalent), as applicable
   ii. Therapeutic Goods Administration (TGA) special access scheme (SAS) notification **(required)**
   iii. Bicon Permit from the Department of Agriculture for importation of phage products from international suppliers, if applicable
   iv. Material Transfer Agreement (MTA) that may be required from third-party suppliers of phage products, if applicable
   v. Licence to handle and administer genetically modified organisms (GMOs) from the Office of the Gene Technology Regulator, if applicable
   vi. Any other local requirements as determined by the primary medical professional caring for the patient (Primary Clinician) or hospital/network Executive board

Selection and sourcing of the phage product to be administered will be based on clinical criteria and will not constitute a research activity.

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10.2 **Exclusion criteria for Participants:**

1. Participant, parent or guardian or person responsible has not provided informed consent.
2. Participant unable or unlikely to adhere to schedule of monitoring and follow-up

11. INTERVENTIONS

11A INTERVENTIONS

11.1 Summary of research activities

This protocol will be embedded within standard clinical care. The following table provides a summary of research activities and standard clinical activities that will be occurring simultaneously.

Table 1: Summary research and clinical activities that occur during phage therapy

Clinical activities	**Research activities**
Decision to access phage therapy	Confirming eligibility of patients to participate, including ensuring suitable phage(s) product (with lytic activity against the target pathogen/s) is available.
Choice of appropriate phage to be administered Route and duration of phage therapy*	Route and duration of phage therapy* Dosing schedule and dose adjustments
Regulatory oversight including TGA SAS notification, hospital drug committee/executive approval (see section 10.1)	Human research ethics committee (HREC) and site-specific approvals
All other healthcare decisions including adjunctive antibiotics, other medications, investigations for underlying health conditions, allied health interventions and standard of care for monitoring investigations for infections.	Schedule of monitoring **relevant to phage therapy**
Frequency of follow-up visits and ongoing monitoring.	Collection of data regarding adverse events during phage therapy and follow-up
	Systematic collection of data to create a registry of phage use in Australia

* Route and duration of phage therapy will be a joint decision between the site PI (research activity) and the Primary Clinician (standard clinical activity)

11.2 Participant identification and referral pathway

Patients for whom phage therapy may be suitable can be identified by any medical professional caring for the patient (Primary Clinician). Each case must be discussed with and approved by at least one infectious diseases specialist who is relevantly clinically privileged at that recruitment site, and who must ensure optimised anti-infective therapy and infection source control. This “approval” can be by

consultation with an infectious diseases specialist or **can** be undertaken by the Primary Clinician if they are so qualified. A second specialist (site PI) must approve and take responsibility for the phage therapy administered according to this protocol. If the available site PI is operating outside their usual professional scope of practice (for example, non-infectious disease specialist) and cannot reasonably take that clinical responsibility, the case should be referred to a suitably experienced PI operating within their normal scope of practice, including by telemedicine consultation, according to the normal rules that govern such consultations. If the site PI is the Primary Clinician, the case must be discussed with one other infectious disease specialist at the site, or at a referral centre. If all inclusion criteria can be met for the patient to be included, the following data should be obtained and entered on the study REDCap database (electronic case report form; eCRF). *****Minimum Data Requirements.

11.2.1 Screening logs

Site PIs should maintain a record of all patients referred for inclusion in the trial including the outcome of assessment for eligibility. Screening for eligibility will occur prior to obtaining consent to participate (see also section 10 above). For patients who are not included in the trial, the reason for this should be clearly documented in a screening log:

i) Antibiotic therapy sub-optimal to date/viable alternative antibiotic therapy available
ii) Source control sub-optimal
iii) Polymicrobial infection (>2 key pathogens)
iv) Pathogen driving infectious syndrome not appropriately confirmed
v) Phage product identified unsuitable
    1. Phage activity against target pathogen(s) limited/undocumented
    2. Formulation not safe for clinical use
    3. Regulatory approvals not completed
vi) Patient/parent or guardian/responsible person unable or unlikely to adhere to schedule of treatment and monitoring
vii) Other (specify)

11.3 Pre-treatment work-up/data

11.3.1 Clinical

i) Primary infectious diagnosis (site of infection, confirmed or potential pathogens, presence of prosthetic material etc.)*****

ii) Current/planned/ongoing adjunctive antibiotics*****

iii) Other interventions performed to control the infection (source control)

iv) Additional medical or surgical conditions that directly impact management of the infection (e.g. CF in a patient with lung infection)*

v) Immune suppressive conditions or therapies*

vi) Indication for phage therapy as assessed by clinical team*
    (1) Intolerance of standard therapy due to side effects, and/or
    (2) Failure of standard therapy, and/or

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(3) Extensive drug resistance profile of target pathogen(s), and/or
(4) Other (to be specified)

11.3.2 Microbiology

i) Culture confirmation from site of infection. If the site of infection is not directly accessible, alternative sites may be sampled if it is reasonable to assume these represent pathogen(s) at the site of infection (e.g. blood culture)*

ii) Phage-bacteria matching (degree of lytic activity between selected phage(s) and target pathogen(s)*. This can be performed at the study site if this service is available within an appropriately accredited laboratory, or within Phage Australia partner sites (e.g. at the Westmead Institute for Medical Research, NSW, the Doherty Institute, Victoria, or the Department of Infectious Diseases laboratory at the Alfred Hospital, Victoria), or by third party phage suppliers (including commercial suppliers, either in Australia or internationally). This may be facilitated by external partners such as Phage Directory (https://phage.directory) and will be confirmed by an appropriately accredited Australian laboratory.

iii) Whole genome sequencing (WGS) of main pathogen(s) according to laboratory standard operating procedures (SOP), and identification of prophages.

iv) WGS of phage(s) to be used

v) Phage-bacteria-antibiotic synergy testing according to laboratory SOP. Lytic activity of suitable phage(s) to be tested against the pathogen of interest in the presence of adjunctive antibiotics as determined through consultation between the Primary Clinician and infectious disease specialists with whom the case has been discussed (see section 11.1 and 11.2 above). This can be performed at the study site if this service is available within an appropriately accredited laboratory, or within Phage Australia partner sites (e.g. at the Westmead Institute for Medical Research, NSW, the Doherty Institute, Victoria, or the Department of Infectious Diseases laboratory at the Alfred Hospital, Victoria).

11.4 Treatment and Monitoring Protocols

The specific phage product to be administered will be determined jointly by the Primary Clinician and Infectious Disease(s) specialist involved in the care of the patient, including consultation with the site PI responsible for oversight of phage therapy according to this protocol (see sections 10.1 and 11.2).

The optimal duration and route of administration of phage will be determined individually for each patient by the PI involved (see section 11.2 above) and will depend on the site of infection, confirmed or suspected pathogens, patient factors (e.g. immune compromise, IV access) and availability of phage products (e.g. formulation, purification).

For intravenously administered phage, the dose will be determined by the endotoxin level of the phage product, keeping below the accepted human pyrogenic threshold of 5 EU/kg per dose set by the United States Food and Drug Administration (FDA). Within this limit, the aim is to administer approximately 10^9 plaque forming units (pfu) of the phage at each dose. 10^9 pfu/dose is aimed to

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achieve a multiplicity of infection (MOI; number of phage virions per bacterial target) of 10-100 per bacterium in the bloodstream for common bacteraemias.

If multiple routes of administration are used, the total dose administered at the same time must not exceed the endotoxin limit outlined above. For enteral, aerosolised/nebulised or topical administration of phage a dose limit has not been determined but is likely to be considerably higher. If a non-intravenous route is selected as the primary route of administration (not just as an adjunct to IV administration), the aim is to administer approximately 10^{10} pfu/dose.

All other care, including adjunctive antibiotics or other medications to be administered, investigations (radiology, laboratory or other), allied health interventions, etc., will be according to routine clinical care and will be determined by the Primary Clinician and the Infectious Disease specialist(s) directly involved in the care of the patient.

11.4.1 Clinical monitoring:

- Full physical examination within 24 hours prior to starting phage therapy (any route of administration)
- Measurement of "vitals" (heart rate [HR], respiratory rate [RR], blood pressure [BP], oxygen saturation, temperature) within 15 minutes prior to, and then 15 and 30 minutes after each dose of phage administered. For patients receiving only topical or nebulised/aerosolised phage products where minimal systemic absorption is expected, only one set of vital signs are required 30 minutes after administration of the phage product.
- Additional clinical monitoring will be according to routine clinical care.

11.4.2 Timing of phage doses:

Frequency of phage doses to be administered for patients receiving 14 days of phage therapy is outlined in Table 2 below. Morning doses should be administered at the same time each day, preferably between 6-10am. On days where twice daily dosing is used, these should be administered 12 hours (+/- 2 hours) apart. Multiple routes of administration may be appropriate for individual patients e.g. IV and nebulised phage therapy for lung infections. For critically unwell patients where urgent control of infection is needed, initial twice daily dosing may be used (i.e. from day 1) after discussion with the site PI. Day of treatment refers to a working day, starting at 6am. Day 0 is any time within 24 hours prior to first dose of phage.

Invasive routes of administration (e.g. intra-articular or endobronchial), should be considered additional to the primary route of administration (e.g. IV) with timing and frequency of such administration determined by the Primary Clinician and the infectious disease specialists/PI involved in the care of the patient.

For patients planned to receive phage therapy for longer than 14 days, dosing frequency will be determined taking into consideration practical limitations. For example, long-term hospital in the home administration of phage therapy may only be possible once daily.

For patients receiving only non-systemically administered phage products where minimal systemic distribution (i.e. viremic dissemination) is expected phage(s) should be administered once daily throughout the course to facilitate outpatient setting administration. The timing of non-systemic

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administration will be determined by the Primary Clinician and the infectious disease specialists/PI involved in the care of the patient.

11.4.3 Laboratory monitoring:

Table 2 outlines laboratory monitoring required for the first 4 weeks in patients receiving 14 days of intravenous phage therapy (majority of participants). Patients receiving oral/enteral therapy should also follow this protocol. Each of the tests listed are included in the Minimum Data Requirements for phage therapy. If it is not practical to obtain samples according to this protocol (e.g. difficult to access site of infection, difficult venous access in young children) this should be discussed with the site PI and reasons documented in the eCRF.

If abnormalities are detected (including worsening of prior abnormalities) on any blood sample that were not present at baseline, these should be followed-up beyond the intervention period (day 0-29) until resolution with frequency of monitoring determined by the Primary Clinician and the infectious disease specialists involved in the care of the patient.

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Table 2: Treatment and laboratory monitoring protocol for standard 14-day intravenous/oral phage therapy

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	29
Phage doses		OD	OD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD		
CRP	X		X		X				X							X	X
ESR	X				X				X							X	X
Procalcitonin (if available)	X		X		X				X							X	
FBC	X		X		X				X			X				X	X
Lymphocyte subsets	X		X		If lymphopenic[£]				If lymphopenic			If lymphopenic				If lymphopenic	If lymphopenic
LFTs	X		X		X				X							X	X
UECs	X		X		X				X							X	X
Phage antibodies*	X											X				X	X
Total IgG	X															X	X
C3, C4, CH50	X															X	X
Cultures**	X				X				X							X	X
Phage susceptibility***	X				X				X							X	X
Kinetics****			X		X				X			X				X	X
Transcriptomics and metagenomics	X		X		X				X			X				X	X

Day 0 – up to 24 hours prior to first dose of phage; OD – once daily; BD – twice daily; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; FBC – full blood count; LFTs – liver function tests; EUCs – electrolytes, urea, creatinine; IgG – total immunoglobulin G; C3, C4, CH50 – complement levels.

Tests in bold are those available in routine clinical laboratories and considered standard of care for monitoring treatment of infections. Although the timing for these tests has been specified according to this protocol to enable standardisation of data collection and analysis, the tests can and should be performed in an appropriately accredited clinical laboratory linked to the recruitment site, as per usual care.

* Phage antibodies - antibodies against relevant therapeutic phages, measured in order to define risk of antibody-mediated neutralisation and/or immune responses. This can be performed at the study site if this service is available within an appropriately accredited laboratory according to SOP, or within Phage Australia partner sites (e.g. at the Westmead Institute for Medical Research, NSW, the Doherty Institute, Victoria, or the Department of Infectious Diseases laboratory at the Alfred Hospital, Victoria), or by third party phage suppliers (including commercial suppliers, either in Australia or internationally).

** For invasive samples baseline (day 0) and post-treatment (day 15) samples are sufficient for monitoring. For patients known or suspected to be bacteraemic at the start of phage therapy, blood cultures should be obtained daily until bacteraemia resolves.

*** All positive cultures of the target pathogen(s) that are isolated after day 1 should be reassessed for ongoing phage-susceptibility, and should have whole genome sequencing performed according to lab SOP.

**** Kinetics - see details in Table 4 and section 11.4.6

£ Lymphopenia will be defined as <1.0 x 10^9/L in individuals aged >12 years, <1.5 x10^9/L in children aged 1-12 years, <4.0 x10^9/L for infants aged 1-12 months, and <3.0 x10^9/L for neonates <1 month.

11.4.4 Ongoing monitoring for long-term systemic phage therapy

For patients planned to receive long-term phage therapy (longer than 14 days), ongoing monitoring will be performed on a monthly basis until 1 month after the end of phage therapy. The following samples and tests will be required for ongoing monthly monitoring for patients receiving >14 days of phage therapy.

- **C-reactive protein**
- **Full blood count**
- **Liver function tests**
- **Urea, creatinine and electrolytes**
- **Total IgG**
- **Complement levels: C3, C4, CH50**
- **Relevant microbiological cultures**
- Phage susceptibility of ongoing positive cultures - All positive cultures of the target pathogen(s) that are isolated after day 1 should be reassessed for ongoing phage-susceptibility, and should have whole genome sequencing performed according to lab SOP.

- Kinetics - see details in Table 4 and section 11.4.6
- Transcriptomics and metagenomics
- Anti-phage antibodies – measured according to lab SOP.

Tests in bold are those available in routine clinical laboratories and considered standard of care for monitoring treatment of infections. These tests can and should be performed in an appropriately accredited clinical laboratory linked to the recruitment site, as per usual care.

11.4.5 Laboratory monitoring for non-systemic therapy

For patients receiving only non-systemically administered phage products where minimal systemic distribution is expected (e.g. topical administration to a wound, or nebulised/aerosolised therapy), Table 3 outlines the laboratory monitoring required for the first 4 weeks. Each of the tests listed are included in the Minimum Data Requirements for phage therapy. If it is not practical to obtain samples according to this protocol (e.g. difficult to access site of infection, difficult venous access in young children) this should be discussed with the site PI and reasons documented in the eCRF.

Table 3: Treatment and laboratory monitoring protocol for standard 14-day non-systemic phage therapy

Day	0	1	2	3	4	5-7	8	9-14	15	29
Phage doses		OD	OD	OD	OD	OD	OD	OD		
CRP	X		X		X		X		X	
FBC	X		X		X		X		X	X
LFTs	X						X		X	
UECs	X						X		X	
Phage antibodies	X								X	X
Cultures*	X						X		X	X
Phage susceptibility**	X						X		X	X

OD – once daily; CRP – C-reactive protein; FBC – full blood count; LFTs – liver function tests; EUCs – electrolytes, urea, creatinine.

* For invasive samples baseline (day 0) and post-treatment (day 15) samples are sufficient for monitoring.

** All positive cultures of the target pathogen(s) that are isolated after day 1 should be reassessed for ongoing phage-susceptibility, and should have whole genome sequencing performed according to lab SOP.

Tests in bold are those available in routine clinical laboratories and considered standard of care for monitoring treatment of infections. These tests can and should be performed in an appropriately accredited clinical laboratory linked to the recruitment site, as per usual care.

For patients planned to receive long-term phage therapy (>14 days), ongoing monitoring will be performed on a monthly basis until 1 month after the end of phage therapy. The following samples

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and tests will be required for ongoing monthly monitoring for patients receiving >14 days of non-systemic phage therapy:

- Full blood count - should be performed in an appropriately accredited clinical laboratory linked to the recruitment site, as per usual care.
- Relevant microbiological cultures and phage susceptibility of ongoing positive cultures - All positive cultures of the target pathogen(s) that are isolated after day 1 should be reassessed for ongoing phage-susceptibility, and should have whole genome sequencing performed according to lab SOP.

11.4.6 Timing of blood samples:

All blood samples should be collected in the morning, prior to the first dose of phage, unless otherwise specified (e.g. post-dose samples for kinetics).

"Kinetics bloods" refers to quantification of phage and bacterial loads in patient blood/serum for patients receiving systemic therapy. These should be determined according to SOP by plaque assay (phage) and quantitative polymerase chain reaction (qPCR; phage and bacteria). The timing of kinetics bloods is outlined in Table 4.

Table 4: Timing of blood samples for quantification of phage and bacterial loads ("kinetics bloods") during phage therapy

	Immediately prior to phage dose	30-60 minutes after phage dose	2-3 hours after phage dose
Phage (plaque assay)	X		X
Phage (qPCR)	X	X	X
Bacteria (qPCR)	X	X	X

All 3 time-points (prior to dose, 30 minutes and 2 hours post-dose) should be drawn for "kinetics" on days 2, 4, 8 and 11. On days 15 and 29, only a single blood draw in the morning is required as no further phage doses will be administered.

11.4.7 Microbiological surveillance

Bacterial cultures should be obtained from the site of infection (or representative site) as outlined above. This may include cultures from blood, urine, sputum, swabs or other clinical specimens. For invasive samples other than blood or routine endotracheal aspirates (e.g. bronchoalveolar lavage or surgical biopsies) baseline (day 0) and post-treatment (day 15) samples are sufficient for monitoring. For patients known or suspected to be bacteraemic at the start of phage therapy, blood cultures should be obtained daily until bacteraemia resolves.

Metagenomics will be performed on clinical samples including blood (as well as sputum, urine, faeces or other samples that may have been collected) to investigate changes in the microbiome during treatment. The analysis pipeline will be restricted to non-human genetic material and will only be used to determine the microbiome of various clinical specimens during and after phage therapy, including endogenous phages.

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11.4.8 Quality-of life questionnaire

A quality-of-life questionnaire will be sent to participants via a survey link in REDCap on days 0 (baseline) and 29, 15 days after completion of the 14-day course of phage therapy, and again at 3 and 6 months (from start of phage therapy). For patients receiving longer than 3 months of phage therapy, the survey link will also be sent out at 12 months.

A validated PROMs tool (EQ-5D-5L/EQ-5D-Y) will be used as the basis of the questionnaire (see section 30 in Appendices) and will ask participants to grade various indicators for quality of life before receiving phage therapy, during and after completion of the course of treatment. Responses to the quality-of life- questionnaires will be reviewed by the site PI within 15 days of completion for each participant.

11.4.9 RNA samples

Whole blood should be obtained for analysis of human transcriptome according to SOP, using a panel that includes both innate and adaptive immune response markers. Analysis will be restricted to immune response genes only.

11.4.10 Long-term follow-up

All participants will be followed-up by the Primary Clinician for a minimum of 6 months after completion of phage therapy. Frequency of follow-up visits will be determined by the Primary Clinician based on the underlying clinical condition (according to routine clinical care). As a minimum, patients should be reviewed clinically, with additional investigations (laboratory or radiology) also determined by the Primary Clinician based on the underlying clinical condition (according to routine clinical care).

11B MODIFICATIONS

11.5 Criteria for modifying phage dose:

Evidence of severe immune-related phenomena after infusion may be criteria for cessation of therapy, as for any severe adverse reaction (see section 20 – Adverse Events Monitoring). Decisions regarding cessation of phage therapy will be made by the Primary Clinician in consultation with the PI based on clinical judgement. The reason for cessation of therapy will be documented in the eCRF. Early cessation of phage therapy will not constitute a withdrawal criterion from the trial for a participant (see 11.6 below) and every effort should be made by the PI to continue all other monitoring of the patient as per the protocol to ensure data integrity and safety monitoring.

Phage levels in the blood prior to re-dosing may be used to determine dosage adjustments(*10*). Doses may be adjusted on the basis of qPCR results for bacteria and phage, or plaque assay for phage, in consultation with the PI. Dose adjustment should be considered in the following scenarios, but should also take into consideration clinical response to treatment at the time.

Table 5: Criteria for consideration of changes to phage doses administered daily

Current dosing schedule	Pre-dose serum phage level by plaque assay	Adjustment
Once daily	$<10^2$ pfu/mL	Increase to twice daily dosing

Once daily	$\geq 10^2$ pfu/mL	Continue once daily dosing
Twice daily	$<10^2$ pfu/mL	Continue twice daily dosing
Twice daily	$\geq 10^2$ pfu/mL	Reduce to once daily dosing

For patients receiving a cocktail of more than one phage, dose adjustments of the whole cocktail should be based on results obtained for the phage with the lowest detectable level of circulating viable phage.

11.6 Criteria for withdrawal of participants:

- Withdrawal of consent – a participant or their parent/guardian/person responsible may withdraw consent to participate in the trial at any point. Any data obtained prior to withdrawal of consent will be retained and the reason for withdrawal recorded in the eCRF. Routine clinical care of the patient will continue, and may include ongoing use of phage for clinical purposes. They will be given the options of:
    - Withdrawing from all subsequent monitoring samples and subsequent collection of clinical data
    - Withdrawing from all subsequent monitoring samples but allowing continued collection of clinical data
- Loss to follow-up – if a non-admitted patient is unable to be contacted (e.g. during the period of follow-up) after multiple attempts to contact them, including at least 3 phone calls and a letter mailed to their last known address, they will be assumed to be lost to follow-up. Any data collected up to that time will remain part of the study data.
- Cessation or dose-modification of phage therapy for medical reasons will not be a criterion for withdrawal from the trial and ongoing monitoring of the participant should continue as per the protocol (see section 11.5 above).

11c Ensuring Adherence to the Interventions

The first 14 days of intravenous phage therapy will be administered in hospital by clinical staff (nursing, pharmacy, medical) and monitored as per SOP and good clinical practice (as outlined in section 11a – Interventions). If phage therapy is planned for longer than 14 days, further treatment can be administered via "Hospital in the Home"-like services. Purely topical, aerosolised or oral/enteral administration of phage can occur in the outpatient setting but must be administered by qualified clinical staff (nursing, pharmacy, medical).

All doses of phage therapy administered will be prescribed by a suitably qualified doctor, dispensed from the hospital pharmacy and recorded in standard medical records (electronic or paper). Additionally, this information will be recorded on the eCRF, including modifications to the dosing schedule or early cessation of therapy.

11d Concomitant Care

Normal optimal anti-infective therapy, including optimised antibiotic and surgical therapy (e.g. source control), vouched for and managed by the responsible/consulting ID physician, is a condition of enrolment and should be recorded on the eCRF. Antibiotic synergy and antagonism (with phage/s) will be tested *in vitro* as soon as feasible. *In vitro* findings of antagonistic phage-antibiotic combinations

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will be notified to the responsible clinical team and will necessitate review of phage and/or antibiotic therapy.

All other care, including other medications to be administered, investigations (radiology, laboratory or other), allied health interventions, etc., will be according to routine clinical care and will be determined by the Primary Clinician and the infectious disease specialists directly involved in the care of the patient.

12. Outcomes and Endpoints

Primary outcome: Short-term Safety

We have previously reported the safe use of bacteriophages in severe sepsis and septic shock(*8*) but phage therapy is still classified as experimental. Although there is 100 years of safety data, there are very few definitive randomised clinical trials (RCTs) and previously undetected adverse events may be uncovered by a more rigorous measurement regime, such as we define herein.

Safety and tolerability will be assessed through adverse events (AEs), vital signs, and clinical laboratory assessments. All AEs will be assessed for severity, causality, and seriousness and will be elicited and assessed from the first dosing until 6 months after the last dose of phage. The PI and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or serious AE (SAE) and remain responsible for following up SAEs that are considered related to the phage therapy or study procedures. The PI is responsible for reporting all SAEs that are observed or reported during the trial, regardless of their clinical significance or relatedness to the administered phage.

Safety will be defined by the absence of serious adverse events (SAEs) attributable to study material (phage). As phages do not attack or integrate into mammalian cells, AEs related to phage therapy are predominantly expected during active administration or shortly thereafter. As such the **primary endpoint** for safety assessments will be any SAE attributable to phage therapy occurring from day 1 (first dose of phage administered) until 15 days after completion of therapy (day 29 for patients receiving 14 days of therapy).

Further details regarding assessing and reporting AEs is provided in section 20 – Adverse Events Monitoring.

Secondary outcomes

1) Additional safety outcomes

During the follow-up period (15 days-6 months after the last dose of phage) only non-serious AEs considered by the investigator to be related to phage therapy, or any SAEs will be reported. These will be ascertained through discussion with the Primary Clinician undertaking the 6-month follow-up and/or review of medical records 6 months following the last dose of phage administered.

Secondary safety endpoints will thus be any SAE OR any AE attributable to phage therapy occurring from day 1 (first dose of phage administered) until 6 months after completion of phage therapy.

2) Feasibility

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Feasibility of using a standardised treatment and monitoring protocol will be assessed as the proportion of participants with >80% of Minimum Data Requirements available for analysis. Minimum Data Requirements are outlined in section 11 above and will vary according to course of phage therapy administered. The **feasibility endpoint** will be assessed at least 15 days after completion of phage therapy.

Exploratory outcomes

We have previously demonstrated eradication of pathogens in settings in which it was unexpected and this can be used as a surrogate for clinical efficacy. The heterogeneity of the casemix and the lack of a control arm (in this non-comparative study design) makes clinical efficacy difficult to assess. The data we develop here will allow the best quality design of formal RCTs and help to inform regulatory reviews.

1) Clinical response to phage therapy

Defined as:

1) Cure – no evidence of ongoing infection: resolution of all clinical symptoms and signs of infection, radiological and laboratory parameters of infection, and microbiological clearance of target pathogen from site of infection (excludes any symptoms, signs, or abnormal radiological and laboratory parameters associated with underlying disease)
    a. Without persisting disability
    b. With persisting disability
2) Partial response
    a. Improvement in clinical signs and symptoms, radiological or laboratory parameters of disease, but with evidence that infection is not completely resolved
    b. Stabilisation of previously documented decline in function, but without obvious improvements, and evidence that infection is not completely resolved
3) No response – evidence of ongoing infection with worsening clinical signs and symptoms, radiological or laboratory parameters of disease

Clinical endpoint: Good clinical response will be defined as all participants assessed as "cured" or with "partial response" at least 15 days after completion of phage therapy.

2) Quality-of-life indicators

Participants will be asked to complete a brief quality-of-life questionnaire using a validated PROMs tool (EQ-5D-5L/EQ-5D-Y) (see section 30 in Appendices). This will ask participants to grade various indicators for quality of life before receiving phage therapy, during and after completion of the course of treatment (see section 11.4.8).

Quality-of-life-endpoints: Changes in EQ-5D-5L/EQ-5D-Y results from baseline to each subsequent assessment (day 29, 3, 6 or 12 months after starting phage therapy).

3) Microbiological clearance of target pathogen(s)

As defined by bacterial cultures of relevant sites (see section 11.4.3-11.4.5 above and Tables 2 and 3). Emergence of anti-phage resistance on treatment will be assessed with all positive cultures of the

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target pathogen(s) that are isolated after day 1 of treatment reassessed for ongoing phage-susceptibility.

Microbiological endpoints include:

- Time to clearance (for each infected site) - day of first negative culture, measured from first day of phage therapy, among participants with sustained clearance (at least 2 consecutive negative cultures with no further positive cultures during the intervention and follow-up period)
- Proportion of participants with sustained microbiological clearance by the end of the intervention period (including up to day 29) – defined as at least 2 consecutive negative cultures by day 29.

4) Phage kinetics over time, antibody development, host immune responses

Dosing strategies at first assume simple clearance – that is, the initial volume of distribution is a simple function of phage delivery/penetration and the clearance is a simple function of usual mechanisms (innate effector cells, renal clearance, non-specific inactivation of viral particles in different body fluids and compartments). However, both (i) phage amplification in target bacterial populations that change over time (predator-prey dynamic that waxes and wanes in a manner that is unique to every single infection), and (ii) development of acquired immune clearance (e.g. neutralising antibodies, likely to vary with individuals) may influence phage kinetics and dose adjustments may be made in response to diagnostic data(*8, 10*). Finally, the host immune response may vary as a function of all these or be independent of them – and significant beneficial immunomodulation may occur in severe sepsis(*8*) and in chronic sepsis(*10*).

These data are observational in nature but are essential for both safety monitoring and for design of future studies. Phage kinetics and human host immune responses as assessed by transcriptomics will be assessed for patients receiving intravenous/oral phage. Anti-phage neutralising/total antibodies will be measured for all patients receiving phage in any formulation (see sections 11.4.3-11.4.5 above and Tables 2 and 3).

5) Microbiome changes

Metagenomics will be performed on any available clinical samples (blood, sputum, urine, faeces or other samples that may have been collected) to investigate changes in the microbiome during treatment. The analysis pipeline will be restricted to non-human genetic material and will only be used to determine the microbiome of various clinical specimens during and after phage therapy, including endogenous phages.

13. PARTICIPANT TIMELINE

Figure 1: Schedule of enrolment, interventions, and assessments for each participant

	STUDY PERIOD			
	Enrolment	**Intervention**		**Follow-up**
TIMEPOINT	***-4w* to Day 0***	***Day 1-14****	***Day 15-29***	***Day 30-210******

ENROLMENT:				
Eligibility screen	X			
Informed consent	X			
Pre-treatment work-up*	X			
Determination of duration and route of phage administration	X			
PHAGE THERAPY**		X		
MONITORING:***				
Blood and clinical sampling	X	X	X	
Quality-of-life questionnaire		X	X	X
Adverse Event Reporting		X	X	X

* Pre-treatment work-up (as outlined in section 11.3) should be performed within 4 weeks of starting phage therapy. If there is a delay (e.g. time required to source phage, or meet legal or regulatory requirements) pre-treatment work up should be repeated within 4 weeks of starting phage therapy. Any invasive sampling or other assessments that are impractical or unsafe to repeat may be referred to the most recently available investigation.

** For some patients, the duration of phage therapy may be longer than 14 days as determined by the PI/infectious disease specialist. Details of phage therapy administration and monitoring are outlined in section 11.4 above.

*** For patients receiving longer than 14 days of phage therapy, long term follow-up will be for 6 months after completion of phage therapy for safety data. An additional quality-of-life survey at 12 months after completion of phage therapy will also occur for patients receiving longer than 3 months of phage therapy. Details of phage therapy administration and monitoring are outlined in section 11.4 above.

14. SAMPLE SIZE

This is an open-label, single-arm clinical trial. All eligible participants that are identified will be recruited until the trial end-date (31 December 2026). No sample-size calculation has been determined. It is estimated that in the first 2 years of the study, 1-4 participants may be recruited at each study site. In subsequent years, greater numbers of participants may be identified and additional study sites will be open for recruitment. The aim is to have 50-100 participants recruited over the 5 years of the study. A study of at least 50 participants receiving phage therapy will enable the proportion who experience an SAE attributable to phage therapy (SARs) to be estimated with a 95% confidence interval (CI) of maximum width ±15%. If the proportion is low the CI will be narrower, and if no participants in 50 experience any such SAE we can be confident that the rate is not higher than 7% (95% CI of 0-7%).

We expect the rate of SARs to be <5%. If 20% or more participants suffer an SAE attributable to the therapy (SARs) then the treatment may not be considered acceptably safe in its current form. An analysis of the first 30 participants who are followed at least 29 days after commencing treatment has 80% statistical power at 5% one-sided alpha to rule out a rate of 20% or higher if the true rate is 5%

or lower, using an exact one-sample binomial test. If 3 or more of these 30 participants have experienced an SAE attributable to therapy (SAR) then consideration will be given to stopping or modifying the study due to safety concerns. Otherwise, recruitment will continue to the target of 50-100 participants to collect data on other outcomes.

15. Recruitment

Participants will be recruited from any hospital (private or public) which is an eligible study site and where human research ethics committee (HREC), governance and all other regulatory approvals are in place (see section 10 – Eligibility Criteria). Outpatient and inpatient healthcare settings (including hospital-in-the-home-like services) will be supported as appropriate, as defined in section 11.4 above. Regional, remote and rural settings will be supported to participate through usual referral pathways to access secondary and tertiary-level care and specialist services. Site PIs have been selected for their ability to identify potentially eligible patients, as well as their clinical expertise in overseeing phage therapy. The majority of PIs are infectious diseases specialists working in tertiary referral centres where patients with difficult-to-treat infections are referred to. This will ensure optimal recruitment of participants into the trial.

Additional activities of the Phage Australia Network outside of this trial, in areas of continuing medical education, community education and workforce development will also ensure that as many suitable patients are screened for eligibility and recruited into the trial as possible.

Consumer co-design and involvement of Cystic Fibrosis Australia representatives in the TSC will also enable recruitment of participants from this patient group which is usually very successful.

Methods: Data Collection, Management, Analysis

16. Data Collection Methods

16a Data Collection Methods

Study data will be hosted on the REDCap platform on the University of Sydney server, secured, managed, and backed-up centrally. Data management practices will follow the principles of the Australian Code for the Responsible Conduct of Research. All data will be handled in accordance with the University of Sydney data handling guidelines. An external data officer/data architect will be contracted for REDCap database construction. Data entry on the REDCap database will be the responsibility of site PIs or a qualified designee. Overall responsibility for data management and data quality control will be with the Investigator team (site PIs).

16b Retention

Cessation or dose-modification of phage therapy for medical reasons will not be a criterion for withdrawal from the trial and every effort should be made to continue all other monitoring of the patient as per the protocol to ensure data integrity and safety monitoring.

If a participant or their parent/guardian/person responsible withdraws consent to participate in the trial, any data obtained prior to withdrawal of consent will be retained. The Primary Clinician is responsible to ensure at least 6 months of clinical monitoring and follow-up after the last dose of administered phage takes place. All attempts will be made to record 6-month follow-up data (for safety monitoring) if the participant provides ongoing consent for this (see section 11.6 above).

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17. Data Management, Protection and Confidentiality

REDCap is a secure platform endorsed by the University of Sydney and many Australian hospitals for research data collection. The REDCap system is hosted on a server behind the University of Sydney controlled firewall, and access is restricted with multi-factor authentication and encrypted connections. Only Principal Investigators or delegates and treating clinicians will be given access to this project through REDCap. They will be able to log on to the project with their access credentials.

Clinical data will be coded but re-identifiable by treating clinicians when collected on REDCap. The 'Data access group' function on REDCap will be used to ensure that clinicians only have access to re-identifiable data for their own patients, not for patients who they are not treating. PIs will have access to re-identifiable data for all patients at their respective recruitment sites.

The 'User rights' function on REDCap will be used to ensure that all data is de-identified when exported for analysis. No user will be able to create data exports from REDCap that contain identifiable information. Scientists involved in biological investigations and data analysis will not require REDCap access; they will only access de-identified exported datasets as needed. All patient data will be de-identified for analysis and dissemination in papers, reports, or presentations.

As part of the responsibilities assumed by participating in the trial, the PI and their delegated proxies (including site sub-investigators) agree to maintain adequate eCRFs and source documents for participants treated as part of the research under this protocol. Source documents may include laboratory and radiology reports, study site notes from medical records, etc. Data quality will be optimised through use of range checks for data values, and error messages for missing data points. Interim analyses as outlined in section 19b below will also be used to highlight errors in data collection/entry.

Most biological samples collected as part of the trial will be processed in clinical diagnostic laboratories and will be stored, processed and discarded as per routine laboratory procedures for clinical samples. Exceptions include samples obtained for measurement of human transcriptomes, metagenomic analysis, anti-phage antibody measurements and phage and bacterial kinetics (see section 11.4 above). For these non-routine assessments, if biological samples are to be shipped for processing at a laboratory external to the recruitment site or their standard diagnostic referral pathways (e.g. at a state-based referral lab for whole genome sequencing of bacterial isolates), including at non-clinical Medical Research Institutes, they will be coded (using the participant's study code + sample details such as date and time of collection, sample type) prior to shipment, storage and analysis. They will be re-identifiable to the PIs and treating clinicians only.

17a Storage

Any left-over biological samples, that have been coded and de-identified may be retained up to 15 years after the study is completed, or until the youngest participant reaches the age of 25, after which they will be destroyed according to laboratory SOP for handling and disposal of clinical waste.

Clinical data and data pertaining to biological samples will be stored on REDCap for 15 years after the trial is completed and data has been exported for analysis, or until the youngest participant reaches the age of 25. Data exported for analysis will be stored on health servers at recruiting sites and accessed via secure-access computers with password protection.

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Any paper records (e.g. consent forms) will be stored in locked offices at recruiting sites and only accessible by study investigators and will be destroyed by shredding, in keeping with healthcare privacy policies. At this point, all electronic data will be completely de-identified.

18. Statistical Methods

18a Outcomes

The primary outcome of the proportion of participants who experience one or more SAEs attributable to study therapy will be presented with a 95% confidence interval. An interim analysis of safety data will be undertaken after 30 participants have been followed for at least one month after commencing treatment. A final analysis will be undertaken when the last enrolled participant has been followed up for at least 6 months after the last dose of phage.

All trial outcomes will be summarised and presented using standard descriptive statistics: frequencies and percentages for categorical data and mean, standard deviation and range or median, quartiles and range for continuous data and the Kaplan-Meier method for time-to-event variables. Results will be presented overall and by sub-groups. Participants may be sub-grouped according to clinical indication (infectious syndrome), route of administration or phage formulation used, or by patient demographics (e.g. age). Exploratory comparisons for all outcomes between subgroups (e.g. adult vs. paediatric patients; those receiving treatment targeting different groups of organisms [Gram-positive vs. Gram-negative organisms]; or different infectious phenotypes [acute bacteraemia vs. chronic osteoarticular infections]) will use standard statistical methods: t-test, chi-square test, log-rank test and corresponding regression models if applicable.

18b Analysis Population and Missing Data

Safety data will be reported for all participants who received any dose of phage therapy. Clinical response to therapy and microbiological clearance outcomes will be reported for participants who received at least 5 days of phage therapy (cumulative). All other outcomes will be reported for all enrolled participants based on available data with no adjustment for missing data.

Methods: Monitoring

19. Data Monitoring

19a Formal Committee

The primary outcome of interest (safety), as well as data completeness, rates of recruitment and clinical outcome assessments, will be monitored by a formal DSMB as outlined in the DSMB Terms of Reference and section 5d.

19b Interim Analyses

A review of included and excluded participants and reasons for inclusion/exclusion will be undertaken annually by the CPI and at least one of the TSC co-conveners to ensure appropriateness of patient selection. Results of this review will be available for the investigator group and the DSMB and may be used for amending the trial protocol.

An interim safety analysis for the primary outcome will be conducted after 30 participants have been followed for at least one month after commencing treatment. If 3 or more of these participants have

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experienced an SAE attributable to therapy (SAR) then consideration will be given to stopping or modifying the study. Study progress including data on all safety, secondary and exploratory outcomes will be regularly reviewed by the trial steering committee. Reports will be provided 6 monthly and will be available for the investigator group and the DSMB and may be used for amending the trial protocol, external reporting and publication, or early termination of the trial. Final decision to terminate the trial rests with the CPI.

Thereafter, trial results will be analysed at the end of 5 years (end of study).

20. Adverse Event Monitoring

20.1 Definitions

An AE is defined as any untoward medical occurrence in a participant enrolled into this trial regardless of its causal relationship to the administered phage product.

An SAE is defined as any event that

- results in death
- is immediately life threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect

Important medical events that may not result in death, be life threatening, or require hospitalisation may be considered SAEs when, based upon appropriate medical judgment, they may jeopardise the participant or may require medical or surgical intervention to prevent one of the outcomes listed in the definition of an SAE.

A suspected, unexpected, serious adverse reaction (SUSAR) is defined as an AE that is serious in nature (i.e. meets SAE criteria), is at least probably (probably or definitely) related to phage therapy (SAR; see section 20.2.2 below) and is an unexpected reaction based on what is known and has been reported in the literature regarding phage therapy. SUSARs will be adjudicated by site PIs.

20.2 Eliciting, Documenting and Reporting Adverse Events

In accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines, all PIs are responsible for recording and reporting adverse events (AE) observed during and after the study. AEs will be assessed from the first dose of phage until the end of the follow-up period, which is 6 months after the last dose of phage.

All AEs that occur during the intervention period (phage administration) and up to 15 days after the last dose of phage will be will be actively collected and reported irrespective of their seriousness or relatedness to administered phage. While patients are admitted to hospital AEs will be solicited through daily clinical reviews. If the patient is discharged home for any duration between day 15 and day 28, AEs occurring over this time will be sought on day 29. For patients receiving outpatient or hospital-in-the-home style therapy, AEs will be solicited at each outpatient appointment/home visit.

During the follow-up period (15 days-6 months after the last dose of phage) only non-serious AEs considered by the investigator to be related to phage therapy, or any SAEs will be reported. These will

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be ascertained through discussion with the Primary Clinician undertaking the 6-month follow-up and/or review of medical records 6 months following the last dose of phage administered.

All AEs and SAEs will be documented on the AE page in the eCRF.

20.2.1 Assessment of Severity

The severity, or intensity, of an AE refers to the extent to which an AE affects the participant's daily activities. The intensity of the AE will be rated as mild, moderate, or severe using the following criteria:

- Mild: These events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate: These events result in a low level of inconvenience or concern. Moderate events may cause some interference with normal functioning.
- Severe: These events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

20.2.2 Assessment of Causality

The relationship of the administered phage in causing or contributing to an AE will be characterised using the following classification and criteria:

- Unrelated: There is no association between the administered phage and the reported event.
- Unlikely: There is a temporal relationship between the administered phage and the reported event; however, it is more likely that the event could have been produced by the participant's clinical state or other treatment(s) administered.
- Possible: The administered phage caused or contributed to the AE, but it could also have been produced by other factors.
- Probable: A reasonable temporal sequence of the event with phage administration exists and, based on previously reported adverse reactions to therapeutic phage, or judgment based on the investigator's clinical experience, the association of the event with the phage seems likely.
- Definite: A definite causal relationship exists between phage administration and the AE, and other conditions (concurrent illness, progression of disease state, or concurrent medication reaction) do not appear to explain the event. The event reappears or worsens if the phage is readministered.

20.3 Reporting Adverse Events

All AEs reported or observed during the trial will be recorded on the AE page in the eCRF. Information to be collected includes the following:

- Phage product
- Phage dose and date/time of last administration
- Event term (MedDRA preferred term)
- Date/time of onset, including day of phage therapy
- Investigator-specified assessment of severity (section 20.2.1) and relationship to the study drug (section 20.2.2)
- Date/time of resolution of the event, including day of phage therapy
- Seriousness (yes/no)

- Any required treatment or evaluations
- Outcome

Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. Any medical condition that is present at the time that the participant is recruited that deteriorates at any time during the trial, should be recorded as an AE.

The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code all AEs. All AEs will be followed to adequate resolution, or until the investigator deems the event to be chronic/stable or not clinically significant, or the participant is lost to follow-up.

Any abnormal laboratory test results or other safety assessments (e.g., vital signs measurements), including those that worsen from baseline, felt to be clinically significant in the medical judgment of the investigator are to be recorded as AEs or SAEs.

20.3.1 Reporting Serious Adverse Events

Any AE that meets SAE criteria (Section 20.1) must be reported by site investigators to the Sponsor within 48 hours after site personnel first learn of the event. The SAE should be reported by completing an SAE report form (template provided in section 29 of Appendices) and emailed to the Sponsor at:

WSLHD-ResearchOffice@health.nsw.gov.au

All SUSARs should also be reported using the SAE report form and notified to the study Sponsor, the chair of the DSMB and trial CPI within 48 hours after site personnel first learn of the event:

WSLHD-ResearchOffice@health.nsw.gov.au AND

ian.seppelt@sydney.edu.au AND

jonathan.iredell@sydney.edu au

An acknowledgement of receipt of email all notifications will be made within 48 hours, with the report forwarded to the responsible HREC (see section 21 – Research Ethics Approval). If events occur over a weekend/public holiday, up to 96 hours is allowed for submitting reports and for acknowledgements. The sponsor will follow NHMRC guidance on investigating and reporting SUSARS, including reporting to the responsible HREC.

In addition, annual safety reports will be provided to the responsible HREC by the trial CPI or qualified delegate which will include a line listing of all SAEs, SARs and SUSARs.

ETHICS AND DISSEMINATION

21. RESEARCH ETHICS APPROVAL

The trial protocol and all trial documents will be submitted for review by the Sydney Children's Hospitals Network (SCHN) HREC under the National Mutual Agreement (NMA). Additional HREC review may be required for sites that are not covered by the NMA (e.g. private hospitals).

22. Protocol Amendments

All major protocol amendments will be submitted for review by SCHN HREC and will only be implemented after approval is gained from HREC (for all participating sites) and local governance committees for each participating recruitment site. Protocol amendments will also be notified to the relevant trial registries, and the journal to which the protocol will be submitted for peer-review and publication.

23. Consent

In accordance with ICH GCP guidelines, written informed consent will be obtained by the PI or a qualified designee from all potential trial participants or from their person responsible for adults with diminished capacity to provide consent, or from a parent or legal guardian for patients aged younger than 18 years. Templates of the Master Participant Information Sheet and Consent Form (PICF) for adult patients (self-consenting, or by person responsible) and for patients younger than 18 (consent from parent/guardian and young person information sheet) are provided in section 28 of the Appendices. The young person participant information sheet will be provided for all young people aged over 10 years. Only HREC-approved PICF will be used to obtain written informed consent. At least 24 hours will be allowed between providing PICFs and obtaining consent for potential participants to consider the information.

24. Declaration of Interests

All financial and other competing interests for PIs and the Trial Steering Committee will be documented according to the Terms of Reference for the Clinical Stream Committee of Phage Australia.

25. Access to Data

The final trial dataset will be made available to the Investigator group. The investigator team will have ongoing custody of data or research outputs, including any intellectual property ownership, with the CPI having overall responsibility.

26. Ancillary and Post-Trial Care

Ancillary and post-trial care will be according to routine practice and will be the responsibility of the Primary Clinician. If further episodes of infection occur, including relapses or refractory illness that require further courses of phage treatment, these will be treated as separate events and will be assessed for eligibility in the trial as for the initial course of treatment (i.e. one patient can be enrolled in the trial more than once if repeated courses of phage therapy are administered that meet all inclusion and exclusion criteria). Courses of phage therapy should be separated by at least 15 days to allow assessment of primary outcome to occur for each course. The first episode per patient will be used for primary analayses, with additional courses described.

26a Unexpected and Secondary Findings

Unexpected secondary findings are extremely unlikely. Whole blood obtained for analysis of human transcriptomes will only be used to investigate immunological responses to the phage therapy using a panel that includes both innate and adaptive immune response markers. No other human genetic analyses are planned and will not be undertaken unless express HREC approval is obtained via an amendment. Metagenomic analyses will be restricted to non-human genetic material used only to analyse changes in the microbiome (including endogenous phages) during treatment. In the unlikely

Khatami A, *et al. BMJ Open* 2022; 12:e065401. doi: 10.1136/bmjopen-2022-065401

event that abnormal immune responses are detected (e.g. limited or reduced gene expression profiles) that may suggest immune deficiency or dysregulation, the results will be fed back to the Primary Clinician and Infectious Disease specialist involved in the care of the patient so that additional clinical or diagnostic assessments can be undertaken as indicated.

27. Dissemination Policy

27a Trial Results

Following interim and final analyses, trial results will be shared with the Investigator group and may be prepared for scientific publication or presentation. Final results will be submitted for publication in a peer-reviewed scientific journal. Authorship on publications arising from the trial will be determined by the Trial Steering Committee as per the Terms of Reference for the Clinical Stream Committee of Phage Australia. A lay summary of the final trial results will be made available for participants and the public on the Phage Australia website (https://criticalinfection.com/phage-australia/). A link to the website will be provided to all study participants upon recruitment. There are no restrictions on publication rights for any data generated from the study.

As the protocol is embedded in usual clinical practice, participants may expect to be informed of test results, response to treatment etc. from their Primary Clinicians. All routinely available clinical assays will be available for clinicians to discuss with participants. For some of the more specialised tests, the results may or may not be available while the patient is being treated. For example, phage/bacterial kinetics will be available and will be used to make dosing adjustments. As the study is not blinded and non-comparative, research staff or Primary Clinicians may inform participants about these results and explain why dose adjustments are being made. Other results such as transcriptomics and metagenomics will be processed at a later stage. The results of these tests will not be reported individually to participants as they will not be clinically meaningful for individuals; however, all patients have a right to access their health and medical records through their usual clinical teams (Primary Clinician).

27b Reproducible Research

The trial protocol will be submitted for publication in an open-access peer-reviewed journal such as BMJ Open or Trials. Specific consent will be obtained for use of data and left-over samples for other related research and where this is granted, this will be used for research which has been approved by an appropriate HREC and upon reasonable written request to the CPI. The de-identified participant-level dataset will be provided on written request to the CPI when the final trial results are submitted for publication.

Appendices

28. Informed consent Materials

Adult Participant Information Sheet and Consent Form (see separate document)

Person Responsible Participant Information Sheet and Consent Form (see separate document)

Parent/Guardian Participant Information Sheet and Consent Form (see separate document)

Youth Participant Information Sheet (see separate document)

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29. Adverse Event/Serious Adverse Event (SAE) Report Form Template

Adverse Event Reporting Form Template (data to be entered directly onto REDCap)

Date of event:

If still receiving phage therapy, specify day of therapy (day 1 = day of first dose administration):

If last dose of phage administered <72 hours prior, specify time of onset of event in hours since last dose of phage:

If last dose of phage administered at least 72 hours prior, specify time of onset of event in days since last dose of phage:

Which phages has participant received at least 1 dose of prior to onset of the event?

Event term (according MedDRA):

Details of event:

Severity: Mild / Moderate / Severe

Serious: Yes / No

If yes, select seriousness criteria (multiple can be selected):

1) resulted in death

2) was immediately life threatening

3) required inpatient hospitalisation or prolongation of existing hospitalisation

4) resulted in persistent or significant disability/incapacity

5) congenital anomaly/birth defect

6) Other, may jeopardise the participant or may require medical or surgical intervention to prevent one of the outcomes listed above

Related: Unrelated / Unlikely / Possible / Probable / Definite

If Probable or Definite relatedness, select from related to (multiple can be selected):

1) phage(s), specify which if multiple used

2) other trial related procedure (e.g. blood draw)

If the event is Serious, confirm SAE has been reported to the Sponsor (WSLHD-ResearchOffice@health.nsw.gov.au) ☐

If the event is Serious with either Probable or Definite relatedness, confirm SAR has been discussed with site PI ☐

In the opinion of the PI, if the event is Serious with either Probable or Definite relatedness, does it qualify as a SUSAR (Suspected, Unexpected Serious Adverse Reaction)? Yes / No

If yes, confirm SUSAR has been reported to the Sponsor (WSLHD-ResearchOffice@health.nsw.gov.au) AND the chair of the DSMB (ian.seppelt@sydney.edu.au) AND the CPI (jonathan.iredell@sydney.edu.au) ☐

Any required treatments or evaluations:

Final outcome: Resolution / Stabilisation / No longer clinically significant / Participant lost to follow-up

Date of final outcome:

If AE requires reporting to the Sponsor, the chair of the DSMB or the CPI/TSC, please print out a report from REDCap and email as an attachment to the appropriate person.

30. Quality-of-Life Questionnaires

EQ-5D-5L (see separate document)

EQ-5D-Y (see separate document)

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